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Adaptive genetic variability and differentiation of Croatian and Austrian *Quercus robur* L. populations at a drought prone field trial

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Abstract. Provenance trials, where populations of different geographical origin are tested in a common environment (common garden test), are a tool suited to allow the study of intraspecific adaptive genetic variation. Research of pedunculate oak (*Quercus robur* L.) adaptive genetic variability through analyses of populations in common garden tests has a long tradition. However, pedunculated oak populations originating south-eastern from the Alps have been scarcely studied in this way. This study addresses the adaptive genetic variability and differentiation of pedunculate oak populations originating from Austria and Croatia in a provenance/progeny field trial. Studied plants were six years old and were growing at the trial for three years. After two years of unusually low precipitations height and survival were analysed. The total mean height of all plants in the trial was 137.8 cm and ranged from 123.0 cm to 151.8 cm. The overall mean survival rate was rather high (0.85). Mean population survival ranged from 0.64 to 0.94. Individual narrow-sense heritabilities (*hi2*), family mean heritabilities (*hf2*), the coefficients of additive genetic variation (*CVA*) and quantitative genetic differentiation coefficients (*QST*) were calculated. A multivariate regression tree (*MRT*) analysis was used to determine the pattern of genetic differentiation of the populations. Individual heritabilities for height ranged between 0.00 and 0.39. Family mean heritabilities for height were rather low in most populations as well (<0.5). Family mean heritabilities for survival were higher than for height (ranging between 0.00 and 0.77). Calculated *QST* coefficients (0.25 for height and 0.14 for survival) indicated between-population genetic differentiation. The populations were separated into two clusters by MRT analysis regarding a climatic variable, namely Hargreaves' reference evapotranspiration. Populations originating from comparatively more humid habitats were grouped in the first cluster. The first cluster had a lower mean height and survival compared to the second one. The differences between these clusters were highly statistically significant. The observed quantitative genetic differentiation might have been driven by natural selection caused by differences in the relative moisture of the habitats

from which the progeny populations originate. The results suggest ecotypic pattern of the quantitative genetic differentiation among studied populations.
Keywords Slavonian oak, ecotype, natural selection, adaptedness, adaptability, quantitative genetic parameters, survival, height

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Introduction

Pedunculate oak (*Quercus robur* L.) is a widespread European forest tree species that prefers fertile and moist habitats (Ducousso & Bordacs 2003). Moreover, this species is one of the most economically valuable European hardwood tree species, and it is a climax species in forests that harbor high biodiversity.
This study primarily investigates pedunculate oak progeny originating from different populations located at the southern edge of the Pannonian Plain (i.e., Slavonia), which is near the southern border of this species' distribution range. Pedunculate oak that grows in the lowland area between the Drava, Sava and Danube Rivers, from Zagreb to Belgrade, is often called Slavonian oak (Gailing et al. 2007). The largest part of this area is within modern Croatia. Some Hungarian botanists have taxonomically distinguished pedunculate oak from this area as a separate subspecies (Matyas 1970), but other authors have characterized Slavonian oak as a form, variety or ecotype (Bartha 2010). Seeds and seedlings of Slavonian oak were used to establish some of the earliest oak provenance tests, in which this taxon showed specific stability with regard to its vigorous height growth, stem form and branching habit (Cieslar 1923, Koloszár 1987). In addition, seeds and seedlings of pedunculate oak from different parts of this area were used for afforestation in parts of Hungary and Germany during the second half of the 19th and the first half of the 20th century. In these afforested areas, the Slavonian provenance proved to be valuable source of reproductive material because of adaptability and trait stability when growing in new environments (Gailing et al. 2007). A smaller number of the progenies in this study originate from populations in Austria, where pedunculate oak is also near the southern border of its range.

The threats associated with global climate change, including rising temperatures, decreasing precipitation and an increasing frequency of extreme climatic events, are expected to intensify in the wider bioclimatic area of Austria and Croatia (Parry 2007, Lindner et al. 2010). Recent forecasts of changes in the distributions of tree species and forest ecosystems show a strong negative impact of climate change on the economic status of forest owners in the region (Hanewinkel et al. 2013).

Theoretically, pedunculate oak may adapt to stressful environmental changes at the individual, population and plant community levels (Lindner et al. 2010) by phenotypic modifications (i.e. plasticity), through natural selection and by hybridization with related xerophilous species such as sessile oak and pubescent oak (Kremer 2010). Species' capacity to adapt by natural selection inevitably depends on their adaptive genetic variability.

Research on the adaptive genetic variability of pedunculate oak through analyses of pop-

ulations in field trials (common garden tests) has a long tradition in Europe (Cieslar 1923, Krahl-Urban 1959, Koloszár 1987, Kleinschmit 1993). However, populations from the western, central and northern parts of the distribution range have been predominantly studied so far (Baliuckas et al. 2001, Siegismund & Jensen 2001, Baliuckas & Pliura 2003, Jensen & Hansen 2008, Maurer et al. 2000, Arend et al. 2011). The number of populations from the southern and south-eastern parts of the range is disproportionately small among older oak field trials, and this area has been unevenly sampled (e.g. Bogdan et al. 2004). It is assumed that there was a secondary glacial refugium in the area of modern Croatia (Petit et al. 2002) and that pedunculate oak in this area may possess a high level of adaptive genetic variability (Hampe & Petit 2005).

Current knowledge is insufficient regarding the adaptive genetic variability and differentiation of pedunculate oak populations, which are widespread in the area southeast of the Alps, and regarding their potential adaptability to predicted climate change. To address this lack of knowledge, three field trials were established in Croatia in the period between 2008 and 2010 that included a more representative sample of populations in this area.

The aims of this study were to determine: (i) the amount of within-population genetic variability, (ii) the amount of between-population genetic differentiation and (iii) the pattern of between-population genetic differentiation. The above mentioned aims were set regarding height growth and survival under conditions of decreased water availability. This evaluation was possible due to significant decrease in precipitation that impacted the location of one field trial during 2011 and 2012.

Materials and methods

Plant material

In the autumn of 2006, acorns were collected in seventeen Croatian pedunculate oak seed stands and five Austrian stands (Table 1, Figure 1). At each stand, acorns were collected from the ground below a minimum of 20 randomly chosen and presumably genetically different trees, each more than 50 m from any other. Saplings were raised at the nursery of the Croatian Forest Research Institute (N 45° 40’ 03.26’’; E 15° 38’ 18.20’’) for three years. The saplings were used as plant material for the establishment of field trials at three sites in Croatia.

Studied field trial

The studied field trial was established in the spring of 2010 at a site near the village of Koška in Osijek-Baranja County of Croatia (Figure 1; N 45° 31’ 42.47’’, E 18° 19’ 09.89’’). This site is a part of a larger lowland forest complex (approx. 10,000 ha), which is dominated by pedunculate oak forests. Prior to the trial establishment, the particular site was not fully overgrown with oak trees because of failure of natural regeneration and was only sporadically overgrown, mainly with black locust (*Robinia pseudoacacia* L.) and various shrubs. According to the Soil Atlas of Europe (2005), the soil at the site of the trial belongs to the Haplic Gleysol type.

The field trial was established with three-year-old saplings according to a randomized complete block experimental design with three replications (blocks). Saplings were planted over a total area of 3.75 ha in 2.5 by 2.0 m spacing. Each of 22 populations was represented by 20 open-pollinated families per block (saplings originating from a common maternal tree were considered a family), and each family was represented by five saplings planted in linear plots. In total, 6,600 saplings that make up the core of the trial were planted. Additionally, two border rows of saplings were planted around the core (720 saplings in total) to reduce the so-called border effect. All saplings were protected after planting with polypropylene tubes (so-called Tuley’s tubes).

Table 1 Origin of the studied pedunculate oak progeny populations and basic climatic variables of their mother stands (reference period 1981-2009)

Provenance abbreviation	Country	Latitude	Longitude	Elevation (m)	Mean annual temperature (°C)	Mean annual precipitation (mm)	Hagreaves's reference evaporation (mm)
A 1	Austria	48.276944	13.307222	368	9.2	902	739
A 13	Austria	46.724079	15.964765	217	10.3	822	781
A 14	Austria	46.626137	14.349256	440	8.6	890	734
A 4	Austria	48.198689	14.746536	230	9.7	750	777
A 5	Austria	47.932968	12.975401	405	9.3	1172	754
HR 12	Croatia	44.916667	18.833333	85	12	770	903
HR 16	Croatia	45.033333	18.900000	81	12	746	902
HR 160	Croatia	45.133333	18.116667	86	11.5	785	883
HR 163	Croatia	45.183333	17.183333	98	11.8	861	888
HR 203	Croatia	45.833333	16.666667	105	11.3	784	840
HR 317	Croatia	45.433333	16.683333	95	11.5	851	858
HR 318	Croatia	45.433333	16.816667	96	11.6	861	863
HR 330	Croatia	45.674167	16.160556	99	11.1	873	829
HR 368	Croatia	45.266667	16.716667	97	11.6	901	868
HR 387	Croatia	45.550000	15.733333	108	11.9	916	842
HR 389	Croatia	45.483333	15.700000	114	11.9	979	843
HR 577	Croatia	45.350000	17.800000	168	11.2	802	859
HR 58	Croatia	45.733333	18.583333	90	11.8	675	859
HR 609	Croatia	45.347700	13.817360	22	14.9	941	758
HR 627	Croatia	46.150000	17.183333	115	11.5	763	838
HR 88	Croatia	45.566667	18.233333	95	11.7	719	861
HR AM	Croatia	45.095917	18.813122	92	11.8	744	898

Climatic conditions at the trial during the period 2011-2012

The climate in 2011 was officially classified as extremely dry and very hot, and that in 2012 was classified as dry and extremely hot by the Croatian Hydrometeorological Service (DHMZ 2013). The mean air temperature during the growing period (March-October) at the trial site in the reference period 1981-2009 was 16.1 °C, with a mean precipitation of 562 mm. Solely based on these data, the field trial site can be characterized as a relatively dry habitat for pedunculate oak. Moreover, the total precipitation in 2011 was 358.5 mm, of which only 233.8 mm fell during the growing season. The total precipitation in 2012 was 619.7 mm, out of which only 366.3 mm fell during the growing season. Thus, in 2011, the study site received only 42% of the average growing-period precipitation and in 2012, only 65% with respect to the reference period 1981-2009.

According to the first readings of three piezometers that were installed at the trial site (on June 3rd, 2011), the groundwater level was below 2.5 m, and from June 27th, 2011, until the end of 2012, the groundwater level remained below 3 m.

The above-described data clearly show that the saplings in the field trial were exposed to substantial decrease in water availability in 2011 and 2012.

Data collection and statistical analysis

In the fall of 2012, when the plants were six years old (and had grown for three years at the

trial site), the heights of all individuals were measured with 1-cm precision, and the survival of all plants were visually determined and scored.

All statistical analysis was generated using SAS/STAT software, Version 9.3 of the SAS System for Windows x64., Copyright © 2002.-2010. by SAS Institute Inc., Cary, NC, USA. Descriptive statistical analyses were performed using the MEANS procedure. Analyses of variance were performed separately for each population using the MIXED procedure to determine the variance components due to the effects of the blocks, families and block-by-family interactions according to the following linear model (ANOVA model 1) (1): where y_{ijk} – individual value of a trait, μ – overall mean, B_i – fixed effect of the block i, i = 1,2,3, F_j – random effect of the family j, j = 1,2, ... 20, BF_{ij} – random effect of the block-by-family interaction, ε_{ijk} – random error.

Combined analyses of variance across all populations were also performed using the MIXED procedure of the SAS software to determine the variance components due to the effects of blocks, populations, families nested within populations, block-by-population interactions and block-by-family interactions according to the following linear model (ANOVA model 2) (2):

$$y_{ijk} = \mu + B_i + F_j + BF_{ij} + \varepsilon_{ijk} \quad (3)$$

$$y_{ijk} = \mu + B_i + F_j + BF_{ij} + \varepsilon_{ijk}$$

$$y_{ijkl} = \mu + B_i + P_k + F(P)_{jk} + BP_{ik} + BF(P)_{ijk} + \varepsilon_{ijkl}$$

$$y_{ijkl} = \mu + B_i + P_k + F(P)_{jk} + BP_{ik} + BF(P)_{ijk} + \varepsilon_{ijkl}$$

where y_{ijkl} – individual value of a trait, μ – overall mean, B_i – fixed effect of the block i, i = 1,2,3, P_k – random effect of the population k, k = 1,2 ... 22, $F(P)_{jk}$ – random effect of the family j nested within the population k, j = 1,2, ... 20, BP_{ik} – random effect of the block-by-population interaction, $BF(P)_{ijk}$ – random effect of the block-by-family within population interaction, ε_{ijkl} – random error.

Variance components as well as their standard errors were estimated using *Reml* and *covtest* options in proc MIXED. Significance of the variance components was tested by likelihood ratio test.

Estimation of quantitative genetic parameters

Variance components given by the *Reml* method in MIXED procedure were used to calculate the following parameters of quantitative genetic variability: individual narrow-sense heritability (h_i^2), family mean heritability (h_f^2), the coefficient of additive genetic variation (CV_A) and quantitative genetic differentiation (Q_{ST}). Variance component due to families within populations estimates a portion of the total genetic variation within populations. It is a portion attributable to the additive genetic variation (a part of genetic variation between individuals in a population). Heritability (h^2) is defined as the ratio of additive genetic variance to phenotypic variance while CV_A is the coefficient of additive genetic variation (Falconer and Mackay 1996). Thus, h^2 and CV_A may be regarded as measures of within-population genetic variability. On the other hand, quantitative genetic differentiation parameter is commonly used measure of among population genetic differentiation for quantitative traits (Houle 1992, Spitze 1993). We used h^2 and CV_A for comparing amounts of within-population genetic variability of studied populations. Thus, we assumed that larger h^2 and CV_A indicate greater amount of within-population genetic variability while larger Q_{ST} indicate higher amount of between population genetic differences.

These quantitative genetic parameters were calculated assuming half-sib relationship of the open-pollinated progenies according to the following formulas:

$$h_i^2 = \frac{4\sigma_f^2}{\sigma_f^2 + \sigma_{bf}^2 + \sigma_\varepsilon^2}$$

$$h_i^2 = \frac{4\sigma_f^2}{\sigma_f^2+\sigma_{bf}^2+\sigma_\varepsilon^2} \quad (4)$$

$$h_f^2 = \frac{\sigma_f^2}{\sigma_f^2+\left(\sigma_{bf}^2/b\right)+\left(\sigma_\varepsilon^2/nb\right)}$$

$$h_f^2 = \frac{\sigma_f^2}{\sigma_f^2+\left(\sigma_{bf}^2/b\right)+\left(\sigma_\varepsilon^2/nb\right)}$$

where: h_i^2 – individual narrow-sense heritability, h_f^2 – family mean heritability, σ_f^2 – variance component of the family effect (given by the ANOVA model 1), σ_{bf}^2 – variance component of the block-by-family interaction effect (given by the ANOVA model 1), σ_ε^2 – variance component of random errors (given by the ANOVA model 1), n - mean observations per plot, b – number of blocks (3).

$$CV_A = \frac{\sqrt{4\sigma_f^2}}{\overline{X}} \times 100;$$

$$CV_A = \frac{\sqrt{4\sigma_f^2}}{\overline{X}} \times 100; \quad (5)$$

where CV_A – coefficient of additive genetic variation; σ_f^2 – variance component of the family effect; $\overline{X}$ – population arithmetic mean of a trait.

$$Q_{ST} = \frac{\sigma_P^2}{\sigma_P^2+(2V_A)}$$

$$Q_{ST} = \frac{\sigma_P^2}{\sigma_P^2+(2V_A)} \quad (6)$$

where Q_{ST} – parameter of quantitative genetic differentiation; σ_P^2 – variance component of the population effect (given by the ANOVA model 2); V_A – additive genetic variance (given by the ANOVA model 2); ($V_A = 4\sigma_f^2 V_A = 4\sigma_f^2$).

Due to the non-normal distribution of the data, all statistical analyses of survival were carried out on the basis of plot means (three values per family in total). This is a relatively common method for analyzing ordinal variables (Jensen 1993, Jensen 2000). For this reason, only h_f^2 values were calculated for survival.

Standard errors of heritabilities were estimated by the Delta method using estimated parameter estimate covariance matrix given by the proc MIXED using *asycov* option.

Analyzing the pattern of between-population genetic differentiation

A multivariate regression tree (MRT) analysis was used to determine the pattern of genetic differentiation. This is a relatively new statistical method that was first used for the delineation of seed zones (Hamann et al. 2011). MRT analysis was performed using the MVpart package v1.2-6 for the R programming environment (R Development Core Team 2008).

Multivariate regression trees (MRT) are based on the same principles as Classification and Regression Trees (CART) but extended to more than one response variable (De'Ath 2002). MRT can be viewed as a constrained clustering methodology that is suitable for explanation as well as prediction. A set of clusters is grown by repeated binary splits of the genetic dataset (in our case populations). Splits are made using environmental predictor variables as criteria, so that the homogeneity of genetic response variables (i.e., the population means of analyzed phenotypic traits) is maximized. Homogeneity is evaluated as sums of squares of traits around the multivariate mean of observations in a cluster (De'Ath 2002). As in CART, no assumptions are made about the mathematical nature of the relationship between response and predictor variables (Hamann et al. 2011). Various climatic variables related to mother stands from which the progeny populations originated were used as environmental predictor variables. To characterize the long-term climate conditions at the source locations of the provenances, we used interpolated climate data for the 1981-2009 reference period that was generated with the ClimateEU software (Hamann, A., Wang T., Spittlehouse D.L., Murdock T.Q. 2013; ClimateEU, unpublished software package for Europe freely available at http://www.ualberta.ca/~ahamann/data/climateeu.html). Estimation of biologically relevant climate variables, lapse- rate elevation adjustments, and data extraction from

grids for the sample locations were carried out with the ClimateEU software, as well. A detailed explanation of estimation of all available climate variables given by the ClimateEU software can be found in Wang et al. (2012). Dependent variables were the standardized arithmetic population means of height and survival (i.e., we subtracted the overall mean and divided by the standard deviation of the traits so that the population trait means are expressed in units of standard deviations from the overall mean of zero).

Results

Height and survival of the populations

We analysed plant heights at the age of six years, out of which three years were growing at the field trial site. The trial site was exposed to substantially less precipitation in the last two years before measurements as compared to the average in the reference period 1981-2009.

The total mean height of all plants in the trial was 137.8 cm and ranged from 123.0 cm (population A4) to 151.8 cm (population HR12). The overall mean survival rate was surprisingly high (0.85). Population A5 had the lowest mean survival (0.64), and the highest mean survival was exhibited by population HR317 (0.94).

Obviously, the studied populations differed with respect to their mean height and survival. Survival rates were above average in populations originating from eastern Croatia. The above-average populations with respect to height were two Austrian and eastern Croatian populations (Table 2).

Within-population genetic variability

Quantitative genetic parameters that describe within-population genetic variability for survival and height at the age of six years are shown in Table 3. Family mean heritabilities for survival were, on average, higher than those for height. For survival, population A5 was distinguished by the highest heritability, whereas populations HR 368 and HR 627 had moderately high heritabilities. Individual heritabilities for height were low in all populations and were in the range of 0.00 to 0.39. Although family mean heritabilities for height were somewhat higher than the individual heritabilities, (0.00 to 0.53), they were low in most populations as well (<0.5). Nevertheless, populations HR203, HR318 and HR387 exhibited moderately high family mean heritabilities for height.

Genetic parameter estimates for across populations

The variance components and quantitative genetic parameters calculated by ANOVA model 2 are shown in Table 4. The effect of population was statistically significant for both analyzed traits, although more so for height, suggesting a significant genetic differentiation of the populations. Moreover, the calculated coefficients of Q_{ST} indicate between-population genetic differentiation, as well.

The variance components of the family effect were also statistically significant for both traits, especially for survival. Consequently, the calculated value of the coefficient of additive genetic variation was higher for survival than for height.

The statistical significance of the block-by-population interaction effect indicates a certain level of plasticity of the studied populations, which was particularly pronounced for survival. The block-by-family interaction was statistically significant for height but not for survival.

Between-population differentiation pattern

Bar charts in the MRT dendrogram represent groups of populations that are similar in both traits, each bar representing the group average for a different trait. Thus, the studied popu-

Table 2 Origin of the studied pedunculate oak progeny populations and basic climatic variables of their mother stands (reference period 1981-2009)

Population	Mean height ± st. error (cm)	Coefficient of variation	Mean survival ± st. error	Survival range of family means	Coefficient of variation
A1	136.4 ± 1.8	21.2	0.87 ± 0.02	0.40-1.00	19.8
A13	145.4 ± 1.6	18.2	0.86 ± 0.02	0.40-1.00	17.8
A14	145.6 ± 2.0	21.4	0.77 ± 0.03	0.00-1.00	32.8
A4	123.0 ± 2.0	25.9	0.82 ± 0.02	0.40-1.00	20.4
A5	130.8 ± 2.4	25.2	0.64 ± 0.03	0.00-1.00	40.7
HR12	151.8 ± 1.9	20.7	0.90 ± 0.02	0.40-1.00	19.3
HR16	134.9 ± 1.9	23.4	0.90 ± 0.02	0.60-1.00	15.1
HR160	140.9 ± 2.1	24.9	0.89 ± 0.02	0.40-1.00	16.2
HR163	147.4 ± 1.8	20.2	0.87 ± 0.03	0.20-1.00	22.3
HR203	134.9 ± 2.1	24.0	0.81 ± 0.02	0.40-1.00	21.7
HR317	138.7 ± 1.6	19.1	0.94 ± 0.01	0.60-1.00	11.1
HR318	136.8 ± 1.8	20.9	0.87 ± 0.02	0.40-1.00	20.2
HR330	129.5 ± 2.1	25.2	0.84 ± 0.02	0.40-1.00	20.0
HR368	138.4 ± 1.8	21.3	0.91 ± 0.02	0.60-1.00	14.3
HR387	132.7 ± 2.0	23.5	0.78 ± 0.03	0.20-1.00	28.5
HR389	142.2 ± 1.9	21.5	0.86 ± 0.02	0.40-1.00	20.2
HR577	138.6 ± 2.0	23.7	0.89 ± 0.02	0.40-1.00	18.2
HR58	138.9 ± 1.9	22.6	0.93 ±0.02	0.60-1.00	13.2
HR609	129.6 ± 2.2	26.4	0.79 ± 0.03	0.20-1.00	27.1
HR627	129.0 ± 2.2	26.2	0.81 ± 0.03	0.20-1.00	27.8
HR88	135.9 ± 2.0	22.6	0.81 ± 0.03	0.20-1.00	23.9
HRAM	146.3 ± 1.8	19.7	0.85 ± 0.03	0.20-1.00	22.4
Overall	137.8 ± 0.4	23.0	0.85 ± 0.01		22.7

lations were separated into two clusters by MRT analysis with respect to a candidate climatic variable, namely, Hargreaves' reference evapotranspiration (Eref; Figure 1). It can be seen from the Figure 1 that populations in the first cluster had lower survival and height on average (i.e. the bars are below the horizontal line that represents the overall mean), while the second cluster populations had higher survival and height on average (the bars are above the overall mean).

The Eref values were calculated on the basis of temperature and latitude. Thus, higher reference evaporation of a site indicates higher temperatures, higher ranges of extreme monthly temperatures and a more southerly geographical position of the site. This parameter indicates how high the potential evaporation is in a given area (potential evaporation depending on the intensity of solar radiation and temperatures). Thus, a higher Eref indicates the potential dryness of a habitat (especially in cases where plant transpiration at a site cannot be compensated by precipitation and/or groundwater), and vice versa, a lower Eref indicates a potentially wetter habitat.

Hence, populations originating from comparatively more humid habitats (Eref < 842.5) were grouped in the first cluster. Populations grouped in the first cluster had lower mean height (134.9 ± 0.92) and survival (0.80 ± 0.01) when compared to the second cluster of populations (mean height = 141.5 ± 0.96, mean survival = 0.89 ± 0.01). Differences between these clusters were highly statistically significant for both traits ($p < 0.0001$).

Table 3 Variance components (given by the ANOVA model 1), and within-population quantitative genetic parameters for height and survival

Population	$^1\sigma^2_f$	$^2\sigma^2_{b\times f}$	$^3\sigma^2_e$	$^4h^2_f$	$^5h^2_i$	^6CV_A (%)
			Height			
A1	0.0	113.3 ± 52.8*	691.6	0.00	0.00	0.0
A13	29.0 ± 29.0	3.2 ± 36.2	633.0	0.36 ± 0.07	0.17 ± 0.03	6.4
A14	0.0	156.4 ± 81.0*	821.6	0.00	0.00	0.0
A4	45.4 ± 41.1	0.0	977.9	0.36 ± 0.05	0.18 ± 0.02	9.5
A5	0.0	84.1 ± 76.9	1009.2	0.00	0.00	0.0
HR12	21.4 ± 33.9	6.0 ± 51.5	960.4	0.22 ± 0.09	0.09 ± 0.02	5.3
HR16	19.2 ± 50.5	160.4 ± 81.7*	809.4	0.14 ± 0.11	0.08 ± 0.04	5.6
HR160	64.1 ± 55.4	50.4 ± 72.3	1080.2	0.39 ± 0.06	0.21 ± 0.03	9.8
HR163	48.5 ± 52.6	135.5 ± 69.3*	702.1	0.32 ± 0.07	0.22 ± 0.05	8.2
HR203	87.3 ± 60	57.4 ± 64.6	863.3	0.49 ± 0.04	0.35 ± 0.05	12.0
HR317	0.0	0.0	693.7	0.00	0.00	0.0
HR318	63.7 ± 41.0	0.0	764.3	0.51 ± 0.03	0.31 ± 0.04	10.1
HR330	61.6 ± 52.4	33.6 ± 69.3	916.6	0.42 ± 0.06	0.24 ± 0.04	10.5
HR368	0.0	52.7 ± 41.5	717.5	0.00	0.00	0.0
HR387	94.7 ± 61.6	16.3 ± 59.2	865.4	0.53 ± 0.04	0.39 ± 0.06	12.7
HR389	21.9 ± 40.8	66.6 ± 65.3	835.3	0.20 ± 0.10	0.09 ± 0.03	5.7
HR577	0.8 ± 41.3	79.2 ± 72.9	1003.4	0.01 ± 0.15	0.00 ± 0.02	1.1
HR58	0.0	178.4 ± 68.5**	821.6	0.00	0.00	0.0
HR609	45.6 ± 62.9	84.3 ± 93.6	1033.0	0.27 ± 0.10	0.16 ± 0.05	9.0
HR627	25.9 ± 62.1	170.2 ± 91.0*	949.7	0.16 ± 0.11	0.09 ± 0.05	6.8
HR88	0.0	83.1 ± 55.7	853.8	0.00	0.00	0.0
HRAM	0.0	100.8 ± 53.6*	718.3	0.00	0.00	0.0
			Survival			
A1	0.005 ± 0.004	0.000	0.022	0.38 ± 0.07	.	13.6
A13	0.003 ± 4.000	0.000	0.021	0.27 ± 0.09	.	10.4
A14	0.002 ± 0.008	0.002 ± 0.013	0.053	0.09 ± 0.13	.	9.7
A4	0.004 ± 0.004	0.000	0.025	0.30 ± 0.08	.	12.6
A5	0.037 ± 0.016**	0.000	0.031	0.78 ± 0.01	.	52.4
HR12	0.003 ± 0.004	0.000	0.026	0.26 ± 0.09	.	10.8
HR16	0.001 ± 0.003	0.000	0.018	0.11 ± 0.12	.	5.4
HR160	0.000	0.001 ± 0.004	0.021	0.00	.	0.0
HR163	0.001 ± 0.004	0.000 ± 0.001	0.024	0.10 ± 0.13	.	6.0
HR203	0.000	0.001 ± 0.006	0.030	0.00	.	0.0
HR317	0.000 ± 0.002	0.000 ± 0.003	0.011	0.04 ± 0.15	.	2.2
HR318	0.000	0.001 ± 0.006	0.031	0.00	.	0.0
HR330	0.000	0.001 ± 0.005	0.026	0.00	.	0.0
HR368	0.004 ± 0.003	0.000	0.012	0.50 ± 0.05	.	12.1
HR387	0.010 ± 0.008	0.000	0.038	0.43 ± 0.06	.	22.1
HR389	0.000	0.001 ± 0.005	0.027	0.00	.	0.0
HR577	0.000	0.001 ± 0.004	0.023	0.00	.	0.0
HR58	0.000	0.000	0.015	0.00	.	0.0
HR609	0.001 ± 0.005	0.001 ± 0.009	0.038	0.08 ± 0.13	.	7.2
HR627	0.014 ± 0.008	0.000	0.033	0.55 ± 0.04	.	25.1
HR88	0.000	0.001 ± 0.006	0.032	0.00	.	0.0
HRAM	0.000	0.001 ± 0.006	0.031	0.00	.	0.0

Table 3 (continuation)
Note. [1]Variance component of the family effect; [2]Variance component of the block-by-family effect; [3]Variance component of the error; [4]Family mean heritability; [5]Individual narrow-sense heritability; [6]Coefficient of additive genetic variation. Significance: * significant at the 0.05 level; ** significant at the 0.01 level.

Table 4 Variance components and quantitative genetic parameter estimates on the basis of combined ANOVA across populations (ANOVA model 2) for height and survival at the age of six years

Trait	Variance components					$^{1}CV_A$ (%)	$^{2}h_i^2$	$^{3}h_f^2$	$^{4}Q_{ST}$
	$^{5}\sigma^2_P$	$^{6}\sigma^2_{b\times P}$	$^{7}\sigma^2_{f(P)}$	$^{8}\sigma^2_{b\times f(P)}$	$^{9}\sigma^2_e$				
Height	41.8** ± 16.0	11.6* ± 5.6	21.2* ± 9.2	75.5*** ± 14.4	852.2 ± 18.5	5.8	0.09 ± 0.00	0.18 ± 0.00	0.25
Survival	0.0027* ± 0.0014	0.0032*** ± 0.0010	0.0027** ± 0.0010	0	0.0284 ± 0.0014	10.6	-	0.22 ± 0.00	0.14

Note. [1]Coefficient of additive genetic variation, [2]Individual narrow-sense heritability, [3]Family mean heritability, [4]Coefficient of quantitative genetic differentiation, [5]Variance component of the provenance effect, [6]Variance component of the block-by-provenance interaction, [7]Variance component of the family within provenance effect, [8]Variance component of the block.by-family within provenance effect, [9]Variance component of the error. Significance: * significant at the 0.05 level, ** significant at the 0.01 level, *** significant at the 0.001 level.

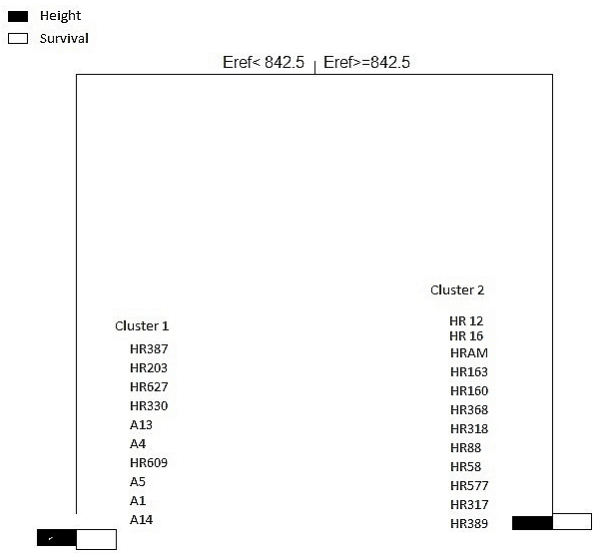

Figure 1
Multivariate regression tree (MRT) analysis of height and survival of studied pedunculate oak populations at the field trial exposed to water deficit. Candidate predictor variable is Hargreaves' reference evapotranspiration (Eref).

Discussion

Height growth and survival

Differences in mean heights among populations at our trial were statistically significant (Table 4). Baliuckas & Pliura (2003) reported lower mean heights of Lithuanian populations, whereas the mean heights of northern European provenances in Danish trials were greater at the same age (Jensen 2000). The mean heights of Polish populations at the age of five years (Barzdajn 1993) were notably lower (even taking into account that those were one year younger than our populations), and the same populations at the age of seven years had mean heights below the mean of our trial (Barzdajn 2008). However, we think

that the mean population heights in our trial were quite low, as these populations mainly originate from and grew in warm conditions of the south where growing period is longer when compared to northern parts of the range. This argument is supported by the fact that the same populations grown in another field trial in Croatia, where it were exposed to less pronounced aridity, showed remarkably greater mean heights compared to those found in the present study (Popovic et al. 2014). Reduced humidity tends to decrease the height growth of pedunculate oak (van Hees, 1997, Thomas & Gausling 2000, Arend et al. 2011); thus, it can be assumed that the relatively low heights of the populations at the studied trial were at least partially a result of the water availability deficit to which the populations were exposed over the period 2011-2012.

Valkonen (2008) reported that the average survival of pedunculate oak saplings at the age of five years under different treatments at different localities of field trials in Finland was above 73%. In comparison with Valkonen's report, the mean survival in our trial was surprisingly high (85%), indicating a relative hardiness of the studied populations to prolonged water deficit. However, differences among the populations were statistically significant (Table 4). Assuming that the mean survival of a population is an indicator of its adaptedness to the habitat conditions in the field trial during the analyzed period, populations with lower than overall average survival may be considered to be less well adapted.

Within-population genetic variability

Differences in the amount of within-population genetic variability were observed (Table 3). The majority of studied populations had low or very low genetic variability for both traits. Populations characterized by a relatively higher level of genetic variability may be considered as more adaptable to specific selection pressures (e.g., drought) than populations with lower genetic variability (Kremer 2010). It should be emphasized that this does not mean that such populations were indeed better adapted to the particular habitat conditions in the field trial during the analysed period; rather, these populations have a higher potential for adaptation, i.e., greater adaptability. For example, population A5 had the highest heritability and CV_A for survival but also exhibited the lowest mean survival. From this result, we can conclude that this population is not, on average, well adapted to the tested habitat conditions but, rather, possesses the potential for adaptation. Thus, by identifying those families within this population that exhibited higher survival rates could produce a subpopulation that was well adapted to the study conditions. Populations HR 627 and HR 387 were other examples of populations that exhibited lower survival but had a higher amount of genetic variability. Thus, these populations could not be considered as well adapted but have relatively higher adaptability to drought in the context of predicted climate change.

Some authors think that CV_A is a better parameter for estimating population adaptability then heritability because CV_A is a measure of variability standardized with an arithmetic mean of a phenotypic trait (Houle 1992). The CV_A values for height of most of the populations were below the reported values of Lithuanian populations of the same age (Baliuckas & Pliura 2003, 2008). Thus, it is possible that the Austrian and Croatian populations, on average, possess a lower level of within-population genetic variability than Lithuanian populations. However, CV_A estimates are strongly dependent on environmental conditions and therefore may vary considerably even within the same population grown at different site conditions. This dependence is also evident in the papers by Baliuckas & Pliura (2003, 2008), in which six populations exhibited significantly different CV_A values for the same traits in different field trials.

Houle (1992) has shown that fitness traits have high coefficients of additive variance despite low heritabilities because of their high

residual variance (i.e., non-additive genetic and environmental variance including error variance) rather than in the depletion of additive genetic variance due to strong directional selection. In our study, the heritabilities and CV_A values were generally positively correlated (i.e., generally, populations with higher heritabilities had higher CV_A values). Therefore, we assume that for many of the studied populations, low additive genetic variance coupled with high residual variance was the cause of their low heritabilities, and conversely, we assume that populations with relatively higher heritabilities maintained a relatively higher additive genetic variance. Of course, it should be acknowledged that lower heritability estimates could have been caused by poor experimental design considering that we analyzed the experiment having no replication in different environments.

Between-population differentiation

Jensen (1993) reported an ecoclinal differentiation pattern of northern European pedunculate oak populations in the field trials in Denmark with respect to flushing and growth cessation. In Jensen's report, more southerly populations (from Netherlands and Germany) were later flushers and ceased growth later, whereas northerly populations (from Norway and Sweden) flushed earlier and ceased growth earlier. The vegetation period length also increased from north to south. Jensen also reported the ecotypic differentiation of Danish local provenances in trials exposed to strong winds and frost. Jensen (2000) revealed a somewhat complex clinal pattern of flushing due to latitude and distance from the ocean. A clinal pattern of pedunculate oak genetic differentiation with respect to the time of budset was reported by Kleinschmit (1993). Southern provenances and those closer to the ocean exhibited later budset, whereas more northern or more continental provenances exhibited earlier budset. In addition, an ecotypic differentiation was exhibited for this trait. Menitsky (1971) reported that a clinal pattern of genetic differentiation was practically unobservable in pedunculate oak. According to Kleinschmit (1993), both ecotype and clinal patterns coexist with regard to phenological traits (flushing, budset). However, a clinal pattern was more obvious for budset. The geographical pattern of pedunculate oak differentiation was not evident for growth, but a relationship between the growth of progeny and climatic conditions in their mother stands was observed. Thus, ecotype differentiation for growth was more plausible (Cieslar 1923, according to Kleinschmit 1993). In contrast to Cieslar's results, Jensen (2000) reported a clear clinal pattern of pedunculate oak differentiation for growth (southern provenances grew faster).

The results of the present study suggest an ecotypic differentiation of the analyzed populations for height and survival. Moreover, the calculated coefficients of Q_{ST} indicate between-population genetic differentiation caused by natural selection, as Q_{ST} values were much higher compared with the Nei coefficients of differentiation that were calculated by analyses of neutral SSR-DNA markers in Croatian pedunculate oak populations (between 0.045 and 0.047) reported by Katičić Bogdan (2012 – Table 14.). The revealed pattern of genetic differentiation may be explained by the humidity of the sites from which populations originated. Generally, populations originating from relatively humid habitats exhibited lower survival rates and heights. However, there were a few exceptions to this general trend. For example, population HR 88 had low average survival even though it originated from a relatively drier habitat. In contrast, Austrian populations A13 and A14 had above-average heights even though they originated from relatively humid habitats. In examining both analyzed traits, the ecotypic differentiation of the populations was clearer for survival because almost all populations (except HR 88) that originated from relatively drier habitats exhibited relatively higher survival rates.

Conclusions

Generally, the studied progeny populations originating from the southeastern part of the pedunculate oak distribution range exhibited surprisingly high mean survival rates after being exposed to two successive years of substantial decrease in water availability at the field trial site. This finding indicates their hardiness, i.e., adaptedness, to relatively long-term arid conditions at the specific trial site. However, differences in survival among populations were statistically significant. A moderate trend of decrease in population mean survival with an increase in deviation among average precipitation at the sites of origin and precipitation at the field trial site was observed. Various amounts of within-population genetic variability for survival and height were estimated. Nevertheless, the vast majority of the populations possessed low or very low genetic variability for both traits, if one examines their quantitative genetic parameters used for describing amount of within population genetic variation. However, it would be premature to conclude that these populations possess low level of adaptability by natural selection, since majority of populations performed relatively high survival rates and heritabilities for height was probably deflated by high random error variances. A high degree of within-population genetic variability was demonstrated for one population although this particular population exhibited the poorest adaptedness to the tested conditions.

The studied populations were significantly genetically differentiated for both traits, although more so for height. The populations exhibiting the highest survival rates originated from the relatively drier habitats of Slavonia in Croatia. The observed quantitative genetic differentiation might have been driven by natural selection caused by differences in the relative moisture of the habitats from which the populations originated. These results suggest an ecotypic pattern of quantitative genetic differentiation.

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References

Arend M., Kuster T., Günthardt-Goerg M.S., Dobbertin M., 2011. Provenance-specific growth responses to drought and air warming in three European oak species (*Quercus robur, Q. petraea* and *Q. pubescens*). Tree Physiology 31:287–297. DOI: 10.1093/treephys/tpr004

Baliuckas V., Lagerström T., Eriksson G., 2001. Within-population variation in juvenile growth rhythm and growth in *Quercus robur* L. and *Fagus sylvatica* L. International Journal of Forest Genetics 8(4): 259-269.

Baliuckas V, Pliura A., 2003. Genetic variation and phenotypic plasticity of *Quercus robur* populations and open-pollinated families in Lithuania. Scandinavian Journal of Forest Research 18: 305–319. DOI: 10.1080/02827580310005153

Baliuckas V, Pliūra A., 2008. Phenogenetic variation pattern in adaptive traits of *Betula pendula, Alnus glutinosa* and *Quercus robur* in Lithuania. Biologija 54: 60–65. DOI: 10.2478/v10054-008-0012-x

Bartha D., 2010. The past, present and future tasks of Hungarian dendrological research. Acta Biologica Hungarica 61: 2–19. DOI: 10.1556/ABiol.61.2010.Suppl.2

Barzdajn W., 1993. Preliminary results of an experiment with Polish provenances of pedunculate oak (*Quercus robur* L.) and sessile oak (*Q. petraea* [Matt] Liebl.). Annales des Sciences Forestières 50(supplement): 222s-227s. DOI: 10.1051/forest:19930721

Barzdajn W., 2008. Comparison of provenance, family and individual heritability of growth traits in pedunculate oak (*Quercus robur* L.) in the family-provenance trial in the Milicz Forest District. Sylwan 5: 52–59.

Bogdan S, Katicic-Trupcevic I, Kajba D., 2004. Genetic variation in growth traits in a *Quercus robur* L. open-pollinated progeny test of the Slavonian Prove-

nance. Silvae Genetica 53: 198-201.
De'Ath G., 2002. Multivariate regression trees: a new technique for modeling species-environment relationships. Ecology 83: 1105-1117.
DHMZ., 2013. Izvješće Hrvatskog Hidrometeorološkog zavoda [Report of the Croatian Hydrometeorological Service]. Ocjena godine. http://klima.hr/ocjene_arhiva.php. (In Croatian).
Ducousso A., Bordacs S., 2003. EUFORGEN Technical Guidelines for genetic conservation and use for pedunculate and sessile oaks (*Quercus robur* and *Q. petraea*). 6 p.
Gailing O., Wachter H., Schmitt H., et al., 2007. Characterization of different provenances of Slavonian pedunculate oaks (*Quercus robur* L.) in Munsterland (Germany) with chloroplast DNA markers: PCR-RFLPs and chloroplast microsatellites. Allgemeine Forst und Jagdzeitung 178(5/6): 85-90.
Hamann A., Gylander T., Chen P., 2011. Developing seed zones and transfer guidelines with multivariate regression trees. Tree Genetics & Genomes 7:399–408. DOI: 10.1007/s11295-010-0341-7
Hampe A., Petit R.J., 2005. Conserving biodiversity under climate change: the rear edge matters. Ecology Letters 8:461–467. DOI: 10.1111/j.1461-0248.2005.00739.x
Van Hees A., 1997. Growth and morphology of pedunculate oak (*Quercus robur* L.) and beech (*Fagus sylvatica* L.) seedlings in relation to shading and drought. Annales des Sciences Forestières 54:9–18. DOI: 10.1051/forest:19970102
Houle D., 1992. Comparing evolvability and variability of quantitative traits. Genetics 130:195–204.
Jensen J.S., 1993. Variation of growth in Danish provenance trials with oak (*Quercus robur* L. and *Quercus petraea* Mattuschka Liebl.). Annales des Sciences Forestières 50(supplement): 203-207. DOI: 10.1051/forest:19930718
Jensen J.S., 2000. Provenance variation in phenotypic traits in *Quercus robur* and *Quercus petraea* in Danish provenance trials. Scandinavian Journal of Forest Research 15:297–308. DOI: 10.1080/028275800447922
Jensen JS, Hansen JK., 2008. Geographical variation in phenology of *Quercus petraea* (Matt.) Liebl and *Quercus robur* L. oak grown in a greenhouse. Scandinavian Journal of Forest Research 23:179–188. DOI: 10.1080/02827580801995331
Jones A., Montanarella L., Jones R., 2005. Soil Atlas of Europe. European Commission. Luxembourg. 128 p.
Katičić Bogdan, I., 2012. Genetska raznolikost hrasta lužnjaka (*Quercus robur* L.) u klonskim sjemenskim planatažama u Hrvatskoj [Genetic diversity of pedunculate oak (*Quercus robur* L.) in clonal seed orchards in Croatia]. Doctoral thesis. University of Zagreb, Faculty of Forestry. 166 p. (In Croatian with English abstract).
Kleinschmit J., 1993. Intraspecific variation of growth and adaptive traits in European oak species. Annales des Sciences Forestières 50(Supplement): 166s-185s. DOI: 10.1051/forest:19930716
Koloszár J., 1987. Die slawonische Eiche in Ungarn [The Slavonic oak in Hungary]. Forst und Holzwirt 11: 293-296.
Krahl-Urban J., 1959. The Oaks. A forestry monograph on *Quercus robur* and *Q. sessitiflora*, 288 pp.
Kremer A., 2010. Evolutionary responses of European oaks to climate change. Irish Forestry 67:53–66.
Lindner M., Maroschek M., Netherer S., Kremer A., Barbati A., Garcia-Gonzalo J., Seidl R., Delzon S., Corona P., Kolström M., Lexer M.J., 2010. Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. Forest Ecology and Management, 259:698–709. DOI: 10.1016/j.foreco.2009.09.023
Matyas V., 1970. New oak taxa of Hungary. Acta Botanica Academiae Scientiarum Hungaricae 16:326–361.
Maurer W.D., Tabel U., König A.O., Stephan B.R., Müller-Starck G., 2000. Provenance trials on *Quercus robur* L. and *Quercus petraea* (Matt.) Liebl. in Rhineland-Palatinate (Germany): preliminary results of phenotypic and genetic surveys. In: Vukelić J., Anić I. (eds.), Proceedings of the IUFRO Unit 2.08. 05 International conference'Oak 2000-Improvement of wood quality and genetic diversity of oaks'. Zagreb, Croatia, 20-25 May 2000, pp. 329–345.
Parry M.L., 2007. Climate Change 2007: impacts, adaptation and vulnerability. Working Group II Contribution to the Fourth Assessment Report of the IPCC Intergovernmental Panel on Climate Change. Cambridge University Press.
Popović M., Ivanković M., Bogdan S., 2014. Variability of height growth and survival of progeny from pedunculate oak (*Quercus robur* L.) seed stands at the field trial 'Jastrebarski lugovi' – first results. Šumarski list 1-2:155-165.
Siegismund H.R., Jensen J.S., 2001. Intrapopulation and interpopulation genetic variation of *Quercus* in Denmark. Scandinavian Journal of Forest Research 16:103–116. DOI: 10.1080/028275801300088143
Spitze K., 1993. Population structure in *Daphnia obtusa*: quantitative genetic and allozymic variation. Genetics 135: 367–374.
Thomas F.M., Gausling T., 2000. Morphological and physiological responses of oak seedlings (*Quercus petraea* and *Q. robur*) to moderate drought. Annals of Forest Science 57:325–333. DOI: 10.1051/forest:2000123
Valkonen S., 2008. Survival and growth of planted and seeded oak (*Quercus robur* L.) seedlings with and without shelters on field afforestation sites in Finland. Forest Ecology and Management 255: 1085–1094. DOI: 10.1016/j.foreco.2007.10.038
Wang T., Hamann A., Spittlehouse D.L., Murdock T.Q., 2012. ClimateWNA-High-resolution spatial climate data for western North America. Journal of Applied Meteorology and Climatology 51:16–29. DOI: 10.1175/JAMC-D-11-043.1